Am J Cancer Res 2026;16(8):3422-3432
www.ajcr.us /ISSN:2156-6976/ajcr0174450

Original Article

HMTA, a natural alkylphenol from *Ardisia virens* Kurz, obstructs autophagic flux to exert anti-pancreatic cancer activity

Yu-Fang Chuang[1], Ying-Ray Lee[2,3,4,5,6], Wan-Chi Tsai[1,6,7,8]

[1]Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung 80708, Taiwan; [2]M. Sc. Program in Tropical Medicine, Kaohsiung Medical University, Kaohsiung 807378, Taiwan; [3]Department of Microbiology and Immunology, College of Medicine, Kaohsiung Medical University, Kaohsiung 807378, Taiwan; [4]Faculty of Post-Baccalaureate Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 807378, Taiwan; [5]Center for Tropical Medicine and Infectious Disease, Kaohsiung Medical University, Kaohsiung 807378, Taiwan; [6]Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung 807378, Taiwan; [7]Drug Development and Value Creation Research Center, Kaohsiung Medical University, Kaohsiung 80708, Taiwan; [8]Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung 80708, Taiwan

Received June 2, 2026; Accepted July 16, 2026; Epub August 15, 2026; Published August 30, 2026

Abstract: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies worldwide due to its aggressive nature and limited therapeutic options. HMTA (4-hydroxy-2-methoxy-6-tridecylphenyl acetate), a natural alkylphenol derived from *Ardisia virens* Kurz, has previously demonstrated anti-tumor potential as a tubulin polymerization inhibitor. However, its impact on the autophagic machinery in pancreatic cancer remains to be elucidated. In this study, we investigated the anti-cancer effects of HMTA across four human pancreatic cancer cell lines (PANC-1, BxPC-3, MIA PaCa-2, and AsPC-1) with varying KRAS mutation statuses. HMTA significantly inhibited cell viability in a dose-dependent manner. Mechanistically, HMTA treatment led to a substantial accumulation of LC3-II and p62/SQSTM1 proteins. Notably, the expression of upstream autophagy-initiating markers, such as ATG5 and Beclin-1, remained unchanged, suggesting a defect in autophagic clearance rather than induction. Using a tandem mRFP-EGFP-LC3 fluorescence reporter assay, we found that HMTA obstructs autophagic flux by preventing the fusion of autophagosomes with lysosomes. Furthermore, the combination of HMTA with the autophagy inhibitor chloroquine resulted in enhanced cytotoxicity, whereas the early-stage inhibitor 3-MA partially rescued HMTA-induced cell death. Our findings demonstrate that HMTA exerts its anti-pancreatic cancer activity mainly through the blockade of autophagic flux, regardless of KRAS background. These results position HMTA as a promising therapeutic candidate for the treatment of PDAC.

Keywords: HMTA, pancreatic ductal adenocarcinoma, autophagic flux, autophagosome-lysosome fusion, alkylphenol

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy characterized by an extremely poor prognosis, with a five-year survival rate remaining below 10% [1, 2]. The silent progression and late-stage diagnosis contribute to its lethality, as most patients present with locally advanced or metastatic disease that is refractory to conventional chemotherapy [3, 4]. Gemcitabine-based regimens and FOLFIRINOX are current standard treatments; however, their efficacy is frequently compromised by innate or acquired chemoresistance [5]. Therefore, identifying novel therapeutic agents that can effectively disrupt PDAC survival signaling is an urgent clinical necessity.

Natural products derived from botanical and animal sources represent a vast reservoir of bioactive scaffolds, many of which possess significant antitumor potential or act as chemosensitizers to enhance traditional therapies [6].

https://doi.org/10.62347/TVYX8770

Among these, species of the genus *Ardisia* (Myrsinaceae) have garnered substantial attention due to their long-standing utility in traditional medicine and as functional food sources [7]. These shrubs and subshrubs are widely distributed across tropical and subtropical Asia, including Taiwan, China, and Southeast Asia [8]. In ethnopharmacological practice, the roots of *Ardisia* species are frequently employed to manage diverse ailments, such as menstrual irregularities, rheumatism, traumatic injuries, and inflammatory pain [8, 9]. Extensive phytochemical profiling has identified *Ardisia* as a prolific source of structurally diverse secondary metabolites-notably alkylphenols-that exhibit potent antioxidant, anti-inflammatory, and antitumor bioactivities [8, 9]. Several isolated alkylphenolic compounds have demonstrated remarkable cytotoxicity against various human cancer cell lines [10-13]; however, the precise molecular targets and the complex signaling networks-particularly those involving intracellular homeostasis-that drive alkylphenol-induced cell death remain largely undefined.

Autophagy is a highly conserved catabolic pathway that maintains cellular homeostasis by sequestering and recycling damaged organelles, misfolded proteins, and microRNAs into double-membranous autophagosomes for lysosomal degradation [14]. In the context of oncology, autophagy functions as a paradoxical "double-edged sword", where its effects are strictly dependent on the tumor stage and cellular environment. While it serves as a tumor suppressor by preventing early-stage malignant transformation in normal cells, it is frequently hijacked by established pancreatic ductal adenocarcinoma (PDAC) cells to buffer metabolic stress, promote longevity, and survive nutrient deprivation [15]. Recent evidence indicates that PDAC tissues exhibit high basal levels of autophagy, which contributes to therapeutic resistance, tumor recurrence, and the maintenance of cancer stem cells [16, 17]. Consequently, the autophagic process has emerged as a critical therapeutic target. Rather than a static state, autophagy is a dynamic "flux" encompassing initiation, elongation, and the final fusion of autophagosomes with lysosomes [18]. Importantly, modern therapeutic strategies aim to disrupt this flux; for instance, traditional inhibitors like chloroquine (CQ) act by blocking autophagosome-lysosome fusion, leading to a lethal accumulation of toxic autophagic vacuoles and triggering apoptosis in PDAC cells [19]. Therefore, a precise, context-specific understanding of how manipulating autophagic flux influences survival and resistance is essential for designing effective, targeted PDAC therapies.

The integrity of the microtubule network is fundamental to the successful execution of autophagic flux, as microtubules serve as the essential "tracks" for the retrograde transport of autophagosomes toward lysosomes for eventual fusion [20, 21]. Consequently, pharmacological agents that disrupt microtubule dynamics often interfere with the late-stage maturation of autophagy. In our previous investigation, we identified HMTA as a potent tubulin polymerization inhibitor that triggers mitotic arrest and subsequent apoptosis in multiple cancer models [12]. While the anti-proliferative effects of HMTA are well-documented, its potential role in modulating the autophagic machinery-specifically whether its microtubule-disrupting property leads to the obstruction of autophagic clearance in pancreatic cancer-remains unexplored.

In the present study, we systematically evaluated the anti-cancer efficacy of HMTA across a panel of human pancreatic cancer cell lines (PANC-1, BxPC-3, MIA PaCa-2, and AsPC-1) encompassing diverse KRAS mutation profiles. Although HMTA-induced tubulin polymerization inhibition, mitotic arrest, and apoptosis have been reported previously [12], whether HMTA affects autophagic flux in pancreatic cancer cells has not been defined. Here, we show that HMTA obstructs autophagic flux by impairing autophagosome-lysosome fusion, leading to the accumulation of LC3-positive autophagosomes and p62/SQSTM1. These findings highlight HMTA-mediated autophagic flux blockade as a previously uncharacterized mechanism underlying its anti-pancreatic cancer activity.

Materials and methods

Cell lines and cell culture

Human pancreatic cancer cell lines (PANC-1, BxPC-3, MIA PaCa-2, and AsPC-1) were purchased from the Bioresource Collection and Research Center in Taiwan. PANC-1 and MIA PaCa-2 cells were cultured in Dulbecco's modi-

fied Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). BxPC-3 and AsPC-1 cells were cultured in RPMI-1640 medium (Gibco BRL) containing 10% FBS, 1% P/S, and 1% sodium pyruvate. All cells were maintained at 37°C in a humidified incubator with 5% CO_2.

Reagents and treatment

HMTA was dissolved in dimethyl sulfoxide (DMSO) to prepare a 10 mg/mL stock solution and stored at -20°C protected from light until use. For cell treatment, the HMTA stock solution was diluted in culture medium to the indicated final concentrations. The final concentration of DMSO was maintained at 0.01% in both control and HMTA-treated groups. HMTA concentrations are presented as μg/mL in the main text, with molar equivalents provided for comparison. Based on the molecular weight of HMTA (364.526 g/mol), 0.25, 0.5, 1, 2, 5, and 10 μg/mL correspond to 0.69, 1.37, 2.74, 5.49, 13.72, and 27.43 μM, respectively.

Cell viability assay

Cells (5×10^3 cells per well) were seeded into 96-well plates and allowed to adhere overnight. The following day, cells were treated with either control medium containing 0.01% dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) or medium supplemented with HMTA at the indicated concentrations and time points. Cell viability was assessed using the 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions [12]. All experiments were performed in triplicate, and data were obtained from three independent experiments.

Cell viability was also evaluated using the trypan blue exclusion assay. Cells were seeded into 24-well plates at a density of 2×10^4 cells/well in medium containing 10% FBS and cultured overnight. The medium was then replaced with 5% FBS-containing medium supplemented with the indicated concentrations of HMTA or DMSO (vehicle control). After 24 h of treatment, cells were collected by trypsinization, combined with the culture medium, and centrifuged at 3,000 rpm for 5 min at 4°C. The cell pellet was resuspended, and an equal volume of cell suspension and 0.4% trypan blue solution (Sigma-Aldrich) was mixed and incubated for 1-3 min. Viable and trypan blue-positive cells were counted using a hemocytometer under an inverted microscope. The percentage of cell death was calculated as the number of trypan blue-positive cells divided by the total number of cells.

Western blotting

To investigate the molecular mechanisms of HMTA, cells were treated with control medium (0.01% DMSO) or with HMTA at the indicated concentrations. After treatment, total cellular proteins were extracted and quantified. Equal amounts of protein were resolved by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% non-fat milk, incubated with specific primary antibodies (all diluted at a range of 1:1,000 to 1:1,500), and then with horseradish peroxidase (HRP)-conjugated secondary antibodies. Immunoreactive bands were detected by enhanced chemiluminescence (ECL) as described previously [12, 22-25]. The primary antibodies used in this study included: mouse anti-LC3 (Santa Cruz; sc-398822), rabbit anti-mTOR (Cell Signaling; 2972), rabbit anti-p-mTOR Ser2481 (Cell Signaling; 2974), rabbit anti-ATG5 (Cell Signaling; 12994), rabbit anti-Beclin-1 (Cell Signaling; 3495), mouse anti-SQSTM1 (p62; Santa Cruz; sc-48402), and mouse anti-β-actin (Santa Cruz; sc-47778).

Immunofluorescence staining

Cells were seeded onto coverslips in 10-cm culture dishes and allowed to adhere overnight at 37°C. After treatment with medium containing either DMSO or HMTA for 24 h, cells were subjected to immunofluorescence staining. Autophagosomes were detected with an anti-LC3 primary antibody (Medical & Biological Laboratories; diluted at 1:200 to 1:500) overnight at 4°C, followed by incubation with fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (GeneTex). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). Autophagosome formation was assessed as previously described [26] using a ZEISS LSM 800 laser-scanning confocal microscope (Oberkochen, Germany).

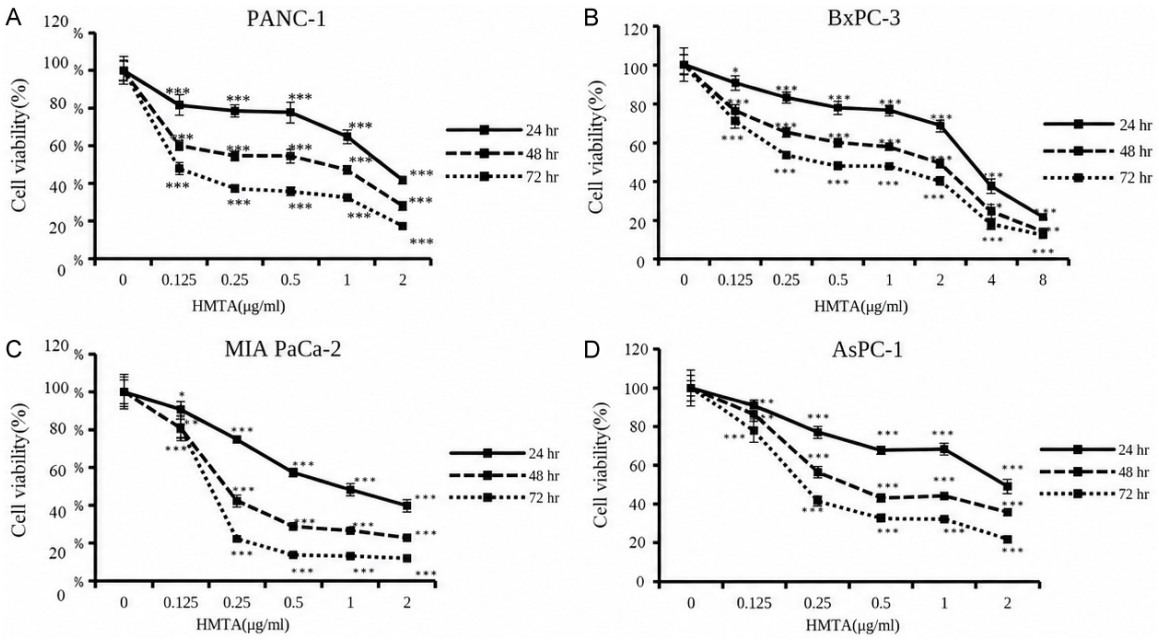

Figure 1. HMTA attenuates the cell viability of human pancreatic cancer cells in a dose- and time-dependent manner. (A) PANC-1, (B) BxPC-3, (C) MIA PaCa-2, and (D) AsPC-1 cells were treated with various concentrations of HMTA for 24, 48, and 72 h. The concentrations of 0.25, 0.5, 1, 2, 5, and 10 μg/mL correspond to 0.69, 1.37, 2.74, 5.49, 13.72, and 27.43 μM, respectively. Cell viability was determined by MTT assay. Data are presented as the mean ± S.D. (n = 6) from three independent experiments. DMSO was used as a vehicle control. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the control group.

Plasmid transfection

To observe autophagosome and autolysosome formation in cells treated with HMTA, cells were transfected with the pmRFP-EGFP-LC3 plasmid (Addgene, Watertown, MA, USA) using Lipofectamine 2000 (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's protocol. After HMTA treatment, autophagosome and autolysosome formation were assessed as previously described [26] by laser-scanning confocal microscopy (ZEISS).

Statistical analysis

All data are presented as the mean ± standard deviation (SD) from at least three independent experiments. Statistical analyses were carried out using SPSS software (version 16.0). For comparisons among multiple groups, one-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's post-hoc test was utilized, whereas comparisons between two groups were performed using the Student's t-test. A p-value of less than 0.05 was considered statistically significant for all analyses.

Results

HMTA attenuates cell viability in human pancreatic cancer cells

To evaluate the anti-cancer efficacy of HMTA, we performed MTT assays across a panel of four human pancreatic cancer cell lines-PANC-1, BxPC-3, MIA PaCa-2, and AsPC-1-representing diverse genetic backgrounds. Cells were exposed to various concentrations of HMTA for 24, 48, and 72 h. As shown in **Figure 1**, HMTA treatment resulted in a dose- and time-dependent reduction in cell viability across all tested lines.

The calculated IC_{50} values (**Table 1**) revealed that sensitivity to HMTA varied among the cell lines, with the potency ranking as follows: MIA PaCa-2 > PANC-1 > AsPC-1 > BxPC-3. Notably, HMTA demonstrated inhibitory effects regardless of KRAS mutation status; it was effective in both KRAS wild-type cells (BxPC-3) and KRAS-mutant cells (PANC-1, AsPC-1, and MIA PaCa-2). Based on these preliminary findings and the representativeness of KRAS status, BxPC-3 and PANC-1 were selected as the pri-

Table 1. Cytotoxic potency (IC_{50} values) of HMTA in various human pancreatic cancer cell lines

IC_{50}(μg/ml)	PANC-1	BxPC-3	MIA PaCa-2	AsPC-1
24 h	1.627±0.096	3.652±1.348	0.894±0.083	1.970±0.179
48 h	0.778±0.082	1.831±0.291	0.225±0.012	0.371±0.042
72 h	0.122±0.011	0.409±0.082	0.206±0.041	0.222±0.016

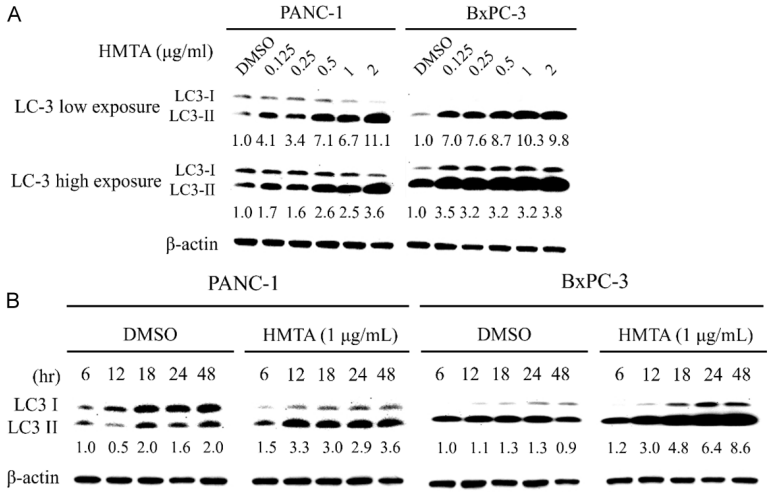

Figure 2. HMTA treatment triggers an increase in LC3-II protein expression. A. PANC-1 and BxPC-3 cells were incubated with indicated concentrations of HMTA for 12 h. B. Cells were treated with 1 μg/mL HMTA for the indicated time intervals. The expression levels of LC3-I and LC3-II were determined by Western blotting. β-actin served as an internal loading control. Relative density values of LC3-II normalized to β-actin are shown below the corresponding blot panels. Densitometric values were normalized to the corresponding DMSO control.

mary models for subsequent mechanistic investigations into HMTA-induced cytotoxicity and autophagic modulation. Specifically, PANC-1 was selected as a KRAS-mutant model with relatively high sensitivity to HMTA, whereas BxPC-3 was selected as a KRAS wild-type model that also exhibited a measurable response to HMTA treatment.

HMTA treatment induces LC3-II accumulation and promotes autophagosome formation

To determine whether HMTA modulates the autophagic process, we first examined the expression of LC3-II, a hallmark protein associated with the formation of autophagosomal membranes. Western blot analysis revealed that treatment with HMTA led to a significant increase in LC3-II levels in both PANC-1 and BxPC-3 cells (**Figure 2A**). Notably, the elevation of LC3-II was observed at relatively low concentrations, reaching a plateau without a strict dose-dependent progression, which may suggest a high potency in obstructing the autophagic pathway. Furthermore, a time-course analysis demonstrated that LC3-II levels increased as early as 12-24 h post-treatment and remained elevated through 48 h, indicating a sustained effect of HMTA on the autophagic machinery (**Figure 2B**).

To further visualize this phenomenon, we performed immunofluorescence staining to detect LC3-positive puncta (representing autophagosomes). As shown in **Figure 3**, HMTA treatment resulted in a marked increase in the number of green fluorescent puncta in both cell lines compared to the DMSO control. To validate the nature of this accumulation, we utilized several pharmacological modulators. The addition of the early-stage autophagy inhibitor 3-MA (10 mM) effectively attenuated the HMTA-induced LC3 puncta, supporting the interpretation that the accumulation was derived from the autophagic pathway. Conversely, combining HMTA with the autophagy inducer rapamycin (200 nM) or the lysosomal inhibitor chloroquine (CQ; 20 μM) did not further augment the number of puncta compared to HMTA treatment alone (**Figure 3**). These results suggest that HMTA treatment triggers the accumulation of autophagosomes, likely by modulating the later stages of the autophagic process.

HMTA does not alter the expression of core autophagy-initiating proteins

To further elucidate whether the observed increase in LC3-II and autophagosome accumulation resulted from the induction of de

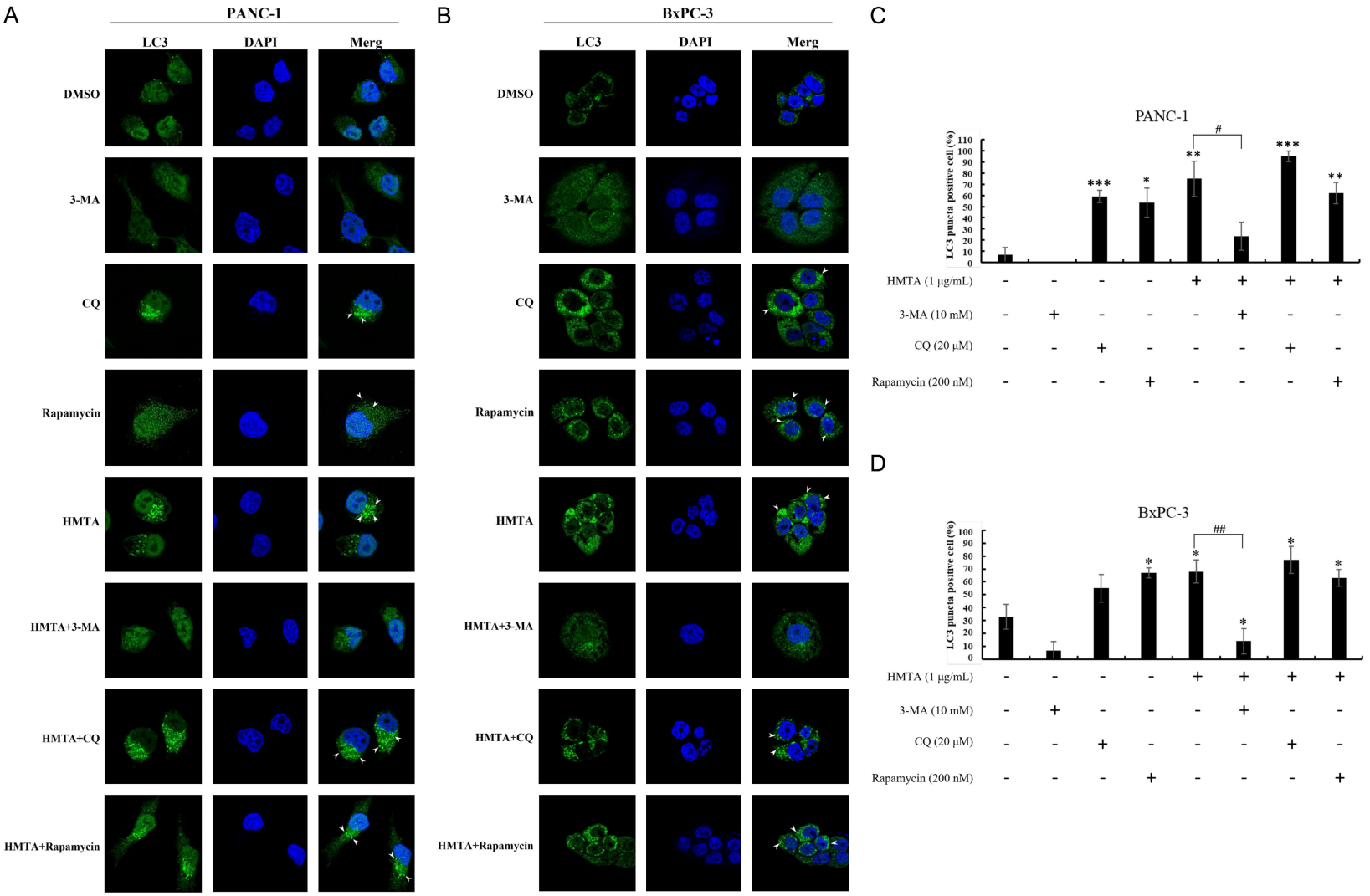

Figure 3. HMTA promotes the accumulation of autophagosomes in pancreatic cancer cells. (A, C) PANC-1 and (B, D) BxPC-3 cells were treated with HMTA (1 μg/mL; 2.74 μM) for 24 h in the presence or absence of 3-MA, chloroquine (CQ), or rapamycin. Autophagosome formation was assessed by immunofluorescence staining using an anti-LC3 antibody and visualized by confocal microscopy. Nuclei were counterstained with DAPI (blue). Arrows indicate the localization of LC3-positive autophagosomes.

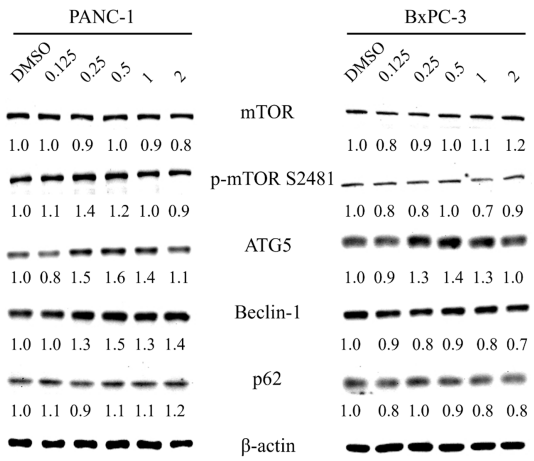

Figure 4. HMTA treatment does not markedly modulate the expression of core autophagy-initiating proteins. PANC-1 and BxPC-3 cells were treated with DMSO or HMTA (μg/mL) for 12 h. The expression levels of mTOR, p-mTOR, ATG5, Beclin-1, and p62 were examined by Western blotting. β-actin was used as a loading control. Relative density values are shown below the corresponding blot panels. mTOR, p-mTOR, p62/SQSTM1, ATG5, and Beclin-1 were normalized to β-actin. Densitometric values were normalized to the corresponding DMSO control.

novo autophagy or the inhibition of autophagic clearance, we examined the expression levels of several core autophagy-related proteins. Western blot analysis demonstrated that HMTA treatment did not significantly modulate the expression of ATG5 or Beclin-1, both of which are essential components for the initiation and elongation of autophagosomes, in either BxPC-3 or PANC-1 cells.

Furthermore, we assessed the activity of the mTOR signaling pathway, a primary negative regulator of autophagy induction. Our results showed that the levels of total mTOR and its activated form, phospho-mTOR (Ser2481), remained largely unchanged following HMTA exposure (**Figure 4**). Additionally, the levels of p62 (SQSTM1), an adapter protein that is typically degraded during successful autophagic flux, did not show a significant decrease, further suggesting that the autophagic degradation process was not enhanced. Semi-quantitative densitometric analysis of the Western blot data showed no consistent HMTA-induced alteration in mTOR/β-actin, p-mTOR/β-actin, ATG5/β-actin, or Beclin-1/β-actin values, while p62/SQSTM1 levels were not reduced after HMTA treatment. These findings indicate that HMTA does not act as a conventional autophagy inducer but instead likely interferes with the downstream machinery of the autophagic pathway.

HMTA obstructs autophagic flux and potentiates cytotoxicity in pancreatic cancer cells

Since HMTA treatment led to autophagosome accumulation without significantly modulating upstream autophagy-initiating proteins, we further investigated whether HMTA blocks autophagic flux. BxPC-3 and PANC-1 cells were transfected with a pmRFP-EGFP-LC3 tandem reporter. In this system, the EGFP signal is sensitive to acidic environments and is quenched upon fusion with lysosomes, whereas the mRFP signal remains stable; thus, autophagosomes appear as yellow puncta (GFP+/RFP+), while autolysosomes appear as red-only puncta (GFP-/RFP+).

Confocal microscopy analysis revealed that control cells exhibited minimal fluorescence, while cells treated with rapamycin (200 nM) showed a significant increase in both yellow and red-only puncta, indicating an active and complete autophagic flux (**Figure 5**). HMTA-treated cells displayed a predominant accumulation of yellow puncta with a notable scarcity of red-only puncta, a pattern similar to that observed in cells treated with the lysosomal inhibitor chloroquine (20 μM) (**Figure 5**). These findings support the interpretation that HMTA disrupts the autophagic process by impairing the fusion of autophagosomes with lysosomes, thereby blocking autophagic flux.

To evaluate the functional consequence of this blockade, we assessed whether the inhibition of autophagic flux contributes to HMTA-mediated cell death using trypan blue exclusion assays. Both HMTA and CQ individually increased the percentage of nonviable cells in PANC-1 and BxPC-3 lines. Importantly, the combination of HMTA and CQ produced a significantly higher rate of cell death compared to either treatment alone (**Figure 6**). Collectively, these data suggest that HMTA-induced anticancer activity is mediated, at least in part, by the blockade of autophagic flux.

Discussion

Pancreatic ductal adenocarcinoma (PDAC) remains a formidable clinical challenge due to its intricate genetic landscape and the rapid

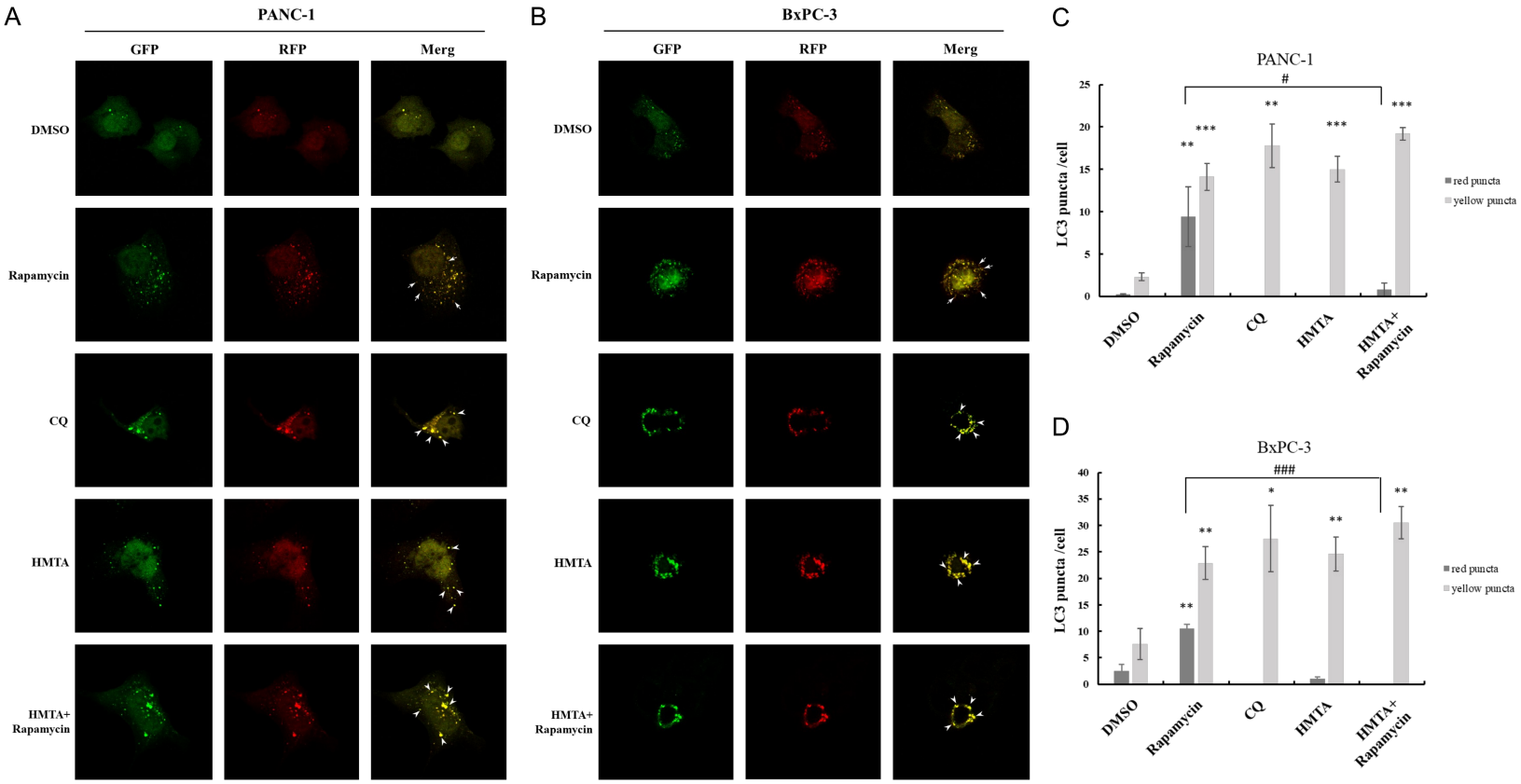

Figure 5. HMTA obstructs autophagic flux by impairing autophagosome-lysosome fusion. (A) PANC-1 and (B) BxPC-3 cells were transfected with the pmRFP-EGFP-LC3 tandem reporter and treated with HMTA (1 μg/mL), Rapamycin (200 nM), or Chloroquine (CQ; 20 μM) for 24 h. In this assay, yellow puncta indicate autophagosomes before lysosomal fusion, in which both EGFP and mRFP signals are retained, whereas red-only puncta indicate autolysosomes after fusion with acidic lysosomes, where the EGFP signal is quenched but the mRFP signal remains stable. Autophagosomes and autolysosomes were visualized by confocal microscopy. (C, D) Quantitative analysis of LC3 puncta per cell. Data represent mean ± S.D. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control. Arrows indicate autophagosomes; arrowheads indicate autolysosomes.

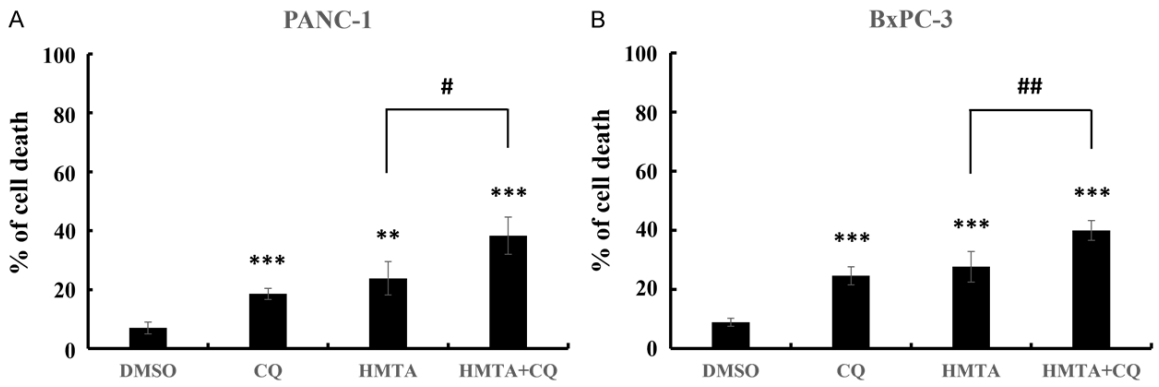

Figure 6. Blockade of autophagic flux by HMTA potentiates cell death in pancreatic cancer cells. (A) PANC-1 and (B) BxPC-3 cells were treated with HMTA and/or chloroquine (CQ) for 24 h. The percentage of cell death was determined by trypan blue exclusion assay. Data are expressed as mean ± S.D. Pairwise comparisons were analyzed by Student's t-test. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control; $\#P < 0.05$, $\#\#P < 0.01$ for the combination group compared with HMTA alone.

development of chemoresistance. In the present study, we provide evidence that HMTA, a natural alkylphenol, exerts anti-pancreatic cancer activity in PDAC cells. Our findings suggest that HMTA-induced cytotoxicity is associated, at least in part, with the blockade of autophagic flux.

A pivotal finding of our study is that HMTA effectively inhibits PDAC cell viability regardless of KRAS mutation status. While oncogenic KRAS mutations (present in nearly 99% of PDAC cases) are known to upregulate basal autophagy to support tumor survival under metabolic stress [16, 27], both KRAS wild-type (BxPC-3) and KRAS-mutant (PANC-1) cells exhibited sensitivity to HMTA-mediated autophagic flux obstruction. This suggests that HMTA may affect a common vulnerability in the autophagic machinery that is essential across diverse genetic backgrounds of pancreatic cancer.

The mechanistic link between HMTA's previously reported activity as a tubulin polymerization inhibitor and the autophagic flux defect observed in the present study remains an important issue for further investigation. Successful autophagic flux is closely associated with the microtubule cytoskeleton, which provides the necessary tracks for the retrograde transport of autophagosomes toward lysosomes [28]. Acetylated microtubules, in particular, are required for the efficient fusion of these vesicles to form autolysosomes [21]. Given that HMTA disrupts microtubule dynamics [12], it is possible that HMTA-mediated tubulin inhibition may contribute to a failure in vacuolar trafficking, resulting in the accumulation of undegraded autophagosomes. This "trapping" of autophagic cargo may impair the recycling of cellular components and increase cellular stress, thereby contributing to HMTA-induced cytotoxicity. However, this microtubule-related mechanism was not directly examined in the present study. Future studies using co-immunofluorescence staining of α-tubulin or acetylated tubulin with LC3 and/or lysosomal markers will be important to further clarify how HMTA affects microtubule-dependent autophagosome trafficking and autophagosome-lysosome fusion.

Furthermore, our study highlights the potential of HMTA in overcoming therapeutic resistance. Autophagy has been implicated in promoting resistance to targeted therapies [29], such as the $KRAS^{G12D}$ inhibitor MRTX1133, by preventing apoptosis through enhanced metabolic pathways [30]. Recent studies have shown that pharmacological inhibition of autophagic flux using agents like chloroquine can sensitize PDAC cells to these inhibitors and even trigger adaptive immune responses [30]. Our observation that combining HMTA with chloroquine significantly enhances cell death suggests a potential benefit of combined late-stage autophagy blockade. Although both agents may affect late-stage autophagy, they may interfere with this process through partially distinct mechanisms. Chloroquine primarily blocks lysosomal acidification and autophagic degradation, whereas HMTA may impair

autophagosome trafficking and autophagosome-lysosome fusion, possibly through microtubule disruption. Therefore, the increased cell death observed with the combination may reflect a more complete impairment of autophagic clearance and increased cellular stress, rather than formally demonstrated pharmacological synergy. This positions HMTA as a promising candidate for combination therapies aimed at circumventing the autophagic survival mechanisms that contribute to resistance in current PDAC treatments.

In conclusion, our data suggest that HMTA exerts its anti-PDAC activity by obstructing autophagosome-lysosome fusion, a process possibly associated with its disruption of the microtubule network. These results provide a possible mechanistic basis for the further evaluation of HMTA as a multifaceted therapeutic agent capable of targeting the unique vulnerabilities of pancreatic12 cancer cells.

Acknowledgements

This work was supported by research grants from the Kaohsiung Medical University Research Foundation (KMU-M113014, KMU-M114015 and KMU-M115007); NPUST-KMU Joint Research Project 115KK027.

Disclosure of conflict of interest

None.

Address correspondence to: Wan-Chi Tsai, Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung 80708, Taiwan. E-mail: wanchi@kmu.edu.tw

References

[1] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024; 74: 229-263.

[2] Klein AP, Brune KA, Petersen GM, Goggins M, Tersmette AC, Offerhaus GJ, Griffin C, Cameron JL, Yeo CJ, Kern S and Hruban RH. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. Cancer Res 2004; 64: 2634-2638.

[3] Hidalgo M, Cascinu S, Kleeff J, Labianca R, Löhr JM, Neoptolemos J, Real FX, Van Laethem JL and Heinemann V. Addressing the challenges of pancreatic cancer: future directions for improving outcomes. Pancreatology 2015; 15: 8-18.

[4] Uson Junior PLS, Kadakia KC and Araujo RLC. Neoadjuvant treatment in resectable pancreatic cancer: Why is upfront surgery so hard to be beaten? World J Clin Oncol 2025; 16: 108955.

[5] Sha M, Gao Y, Yin X, Li X, Liu C and Li S. Engineered exosomes: a promising approach for overcoming challenges in pancreatic cancer therapy. J Nanobiotechnology 2025; 23: 619.

[6] Shi CS, Li JM, Chin CC, Kuo YH, Lee YR and Huang YC. Evodiamine induces cell growth arrest, apoptosis and suppresses tumorigenesis in human urothelial cell carcinoma cells. Anticancer Res 2017; 37: 1149-1159.

[7] Kobayashi H and de Mejia E. The genus Ardisia: a novel source of health-promoting compounds and phytopharmaceuticals. J Ethnopharmacol 2005; 96: 347-354.

[8] Zhu GY, Wong BC, Lu A, Bian ZX, Zhang G, Chen HB, Wong YF, Fong WF and Yang Z. Alkylphenols from the roots of *Ardisia brevicaulis* induce G1 arrest and apoptosis through endoplasmic reticulum stress pathway in human non-small-cell lung cancer cells. Chem Pharm Bull (Tokyo) 2012; 60: 1029-1036.

[9] Zhao F, Hu Y, Chong C, Lu M, Chen L, Kan W, Chen L and Liu H. Ardisiphenol D, a resorcinol derivative identified from *Ardisia brevicaulis*, exerts antitumor effect through inducing apoptosis in human non-small-cell lung cancer A549 cells. Pharm Biol 2014; 52: 797-803.

[10] Chen LP, Zhao F, Wang Y, Zhao LL, Li QP and Liu HW. Antitumor effect of resorcinol derivatives from the roots of *Ardisia brevicaulis* by inducing apoptosis. J Asian Nat Prod Res 2011; 13: 734-743.

[11] Bao L, Wang M, Zhao F, Zhao Y and Liu H. Two new resorcinol derivatives with strong cytotoxicity from the roots of *Ardisia brevicaulis* Diels. Chem Biodivers 2010; 7: 2901-2907.

[12] Yen CH, Lin CJ, Chen PY, Chen YJ, Wei LR, Chen PH, Yeh YC, Wang LH, Chang HS and Tsai WC. Taming pancreatic cancer: *Ardisia virens* Kurz-derived 4-hydroxy-2-methoxy-6-tridecylphenyl acetate as a potent tubulin polymerization inhibitor for targeted pancreatic ductal adenocarcinoma therapy. Int J Med Sci 2025; 22: 651-661.

[13] Chang HS, Lin YJ, Lee SJ, Yang CW, Lin WY, Tsai IL and Chen IS. Cytotoxic alkyl benzoquinones and alkyl phenols from *Ardisia virens*. Phytochemistry 2009; 70: 2064-2071.

[14] Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, Abdelfatah S, Abdoli A, Abel S, Abeliovich H, Abildgaard MH, Abudu YP, Acevedo-Arozena A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)[1]. Autophagy 2021; 17: 1-382.

[15] Jalali P, Shahmoradi A, Samii A, Mazloomnejad R, Hatamnejad MR, Saeed A, Namdar A and Salehi Z. The role of autophagy in cancer: from molecular mechanism to therapeutic window. Front Immunol 2025; 16: 1528230.

[16] Li J, Chen X, Kang R, Zeh H, Klionsky DJ and Tang D. Regulation and function of autophagy in pancreatic cancer. Autophagy 2021; 17: 3275-3296.

[17] Ko YH, Cho YS, Won HS, Jeon EK, An HJ, Hong SU, Park JH and Lee MA. Prognostic significance of autophagy-related protein expression in resected pancreatic ductal adenocarcinoma. Pancreas 2013; 42: 829-835.

[18] Mizushima N. Autophagy: process and function. Genes Dev 2007; 21: 2861-2873.

[19] Loncle C, Molejon MI, Lac S, Tellechea JI, Lomberk G, Gramatica L, Fernandez Zapico MF, Dusetti N, Urrutia R and Iovanna JL. The pancreatitis-associated protein VMP1, a key regulator of inducible autophagy, promotes Kras(G12D)-mediated pancreatic cancer initiation. Cell Death Dis 2016; 7: e2295.

[20] Kaur R, Kaur G, Gill RK, Soni R and Bariwal J. Recent developments in tubulin polymerization inhibitors: an overview. Eur J Med Chem 2014; 87: 89-124.

[21] Xie R, Nguyen S, McKeehan WL and Liu L. Acetylated microtubules are required for fusion of autophagosomes with lysosomes. BMC Cell Biol 2010; 11: 89.

[22] Tung CL, Chao WY, Li YZ, Shen CH, Zhao PW, Chen SH, Wu TY and Lee YR. Ivermectin induces cell cycle arrest and caspase-dependent apoptosis in human urothelial carcinoma cells. Int J Med Sci 2022; 19: 1567-1575.

[23] Chang JM, Wu JY, Chen SH, Chao WY, Chuang HH, Kam KH, Zhao PW, Li YZ, Yen YP and Lee YR. 9-O-terpenyl-substituted berberrubine derivatives suppress tumor migration and increase anti-human non-small-cell lung cancer activity. Int J Mol Sci 2021; 22: 9864.

[24] Chang JM, Kam KH, Chao WY, Zhao PW, Chen SH, Chung HC, Li YZ, Wu JY and Lee YR. Berberine derivatives suppress cellular proliferation and tumorigenesis in vitro in human non-small-cell lung cancer cells. Int J Mol Sci 2020; 21: 4218.

[25] Lee MY, Shi CS, Hsu YC, Huang KJ, Chen SH, Zhao PW, Chung HC, Huang YC and Lee YR. Honokiol is a potential therapeutic agent and has a synergistic effect with 5-FU in human urothelial cell carcinoma cells. Anticancer Res 2019; 39: 6555-6565.

[26] Yu HI, Shen HC, Chen SH, Lim YP, Chuang HH, Tai TS, Kung FP, Lu CH, Hou CY and Lee YR. Autophagy modulation in human thyroid cancer cells following aloperine treatment. Int J Mol Sci 2019; 20: 5315.

[27] Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Fraser BA, Kawaguchi Y, Johann D, Liotta LA, Crawford HC, Putt ME, Jacks T, Wright CV, Hruban RH, Lowy AM and Tuveson DA. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003; 4: 437-450.

[28] Hasanain M, Sahai R, Pandey P, Maheshwari M, Choyal K, Gandhi D, Singh A, Singh K, Mitra K, Datta D and Sarkar J. Microtubule disrupting agent-mediated inhibition of cancer cell growth is associated with blockade of autophagic flux and simultaneous induction of apoptosis. Cell Prolif 2020; 53: e12749.

[29] Li X, Zhou Y, Li Y, Yang L, Ma Y, Peng X, Yang S, Liu J and Li H. Autophagy: a novel mechanism of chemoresistance in cancers. Biomed Pharmacother 2019; 119: 109415.

[30] Han L, Meng L, Liu J, Xie Y, Kang R, Klionsky DJ, Tang D, Jia Y and Dai E. Macroautophagy/autophagy promotes resistance to KRAS(G12D)-targeted therapy through glutathione synthesis. Cancer Lett 2024; 604: 217258.